Studies of selectivity in protein interactions with polymer surfaces

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INTRODUCTION

Materials used for medical applications are required to be compatible with the biological systems in which they are contact over the useful life of the device. There is a huge global effort in biomaterial optimization. A common belief is that the first interactions between a foreign material - such as a polymer used for blood contact applications such as catheters, stents, etc - play an important role in the subsequent evolution of the bio-material interaction. In favorable cases, the biological system either ignores the material or produces an inert 'biofilm' which does not trigger foreign body rejection mechanisms. In unfavorable cases, dangerous consequences can arise, such as clot formation in a blood application. Polyurethanes, which are common in many blood contact applications due to their favorable mechanical properties, phase segregate into hard (typically aromatic-rich) and soft (typically polyether rich) domains. The domain structure at the surface of these materials and its dependence on history and environment is poorly understood, as is whether or not there is a preference for key blood proteins to attach to the hard or soft domains if both are exposed at the surface. We are exploring the use of soft X-ray spectromicroscopy - both scanning transmission X-ray microscopy (STXM) [1] and X-ray photoelectron emission microscopy (X-PEEM) [2] - as a tool for measuring surface domain chemistry of proteins, and for monitoring selectivity in protein interactions with biomaterial polymer surfaces which have lateral chemical differentiation.

RESULTS AND DISCUSSION

Several reports on earlier phases of this project have been published elsewhere [3-5]. We are close to demonstrating all capabilities needed for X-ray microscopy to be a useful tool in this application. In particular, both STXM and X-PEEM can map the substrate domain structure of polyurethanes, and both techniques can detect protein relative to that domain structure at about monolayer amounts. As described below, in our October 2000 run, we were able to demonstrate that STXM can detect monolayer levels of fibrinogen on a phase segregated polyurethane. However, all of the substrates we have used so far have had corrugated surfaces, either due to cutting artifacts in the cryomicrotomed sections used for STXM, or due to inability to make adequately flat polyurethane samples by solvent or spin casting. In the past year we have explored extensively spun-cast blends of polystyrene-polymethylmethacrylate (PS/PMMA) as an alternative, model substrate for protein adsorption on a laterally differentiated hydrophobic/ hydrophilic surface. However, as reported elsewhere [6,7] although the samples are extremely flat, and have suitable domian sizes (>200 nm) all of our PS/PMMA preparations appear to have an outer surface of almost pure PS. We are continuing to work on the PS/PMMA blend chemistry and spectromicroscopy, both to understand the reason for the surface PS, and to try to make a metastable sample with exposed PMMA and PS domains, suitable for the model protein attachment studies. In parallel we have continued to explore aspects of protein adsorption on solvent cast polyurethanes and on microtomed sections. An additional project with strong

connections to this one, is our work with Castner using X-PEEM to characterize protein interactions with artificial surfaces prepared by microcontact printing and self-assembly gold-thiol techniques [8].

An important result of the past year has been demonstration of the ability to detect protein adsorption on fully hydrated polymer surfaces using STXM and wet cell techniques. This is a very important extension since the wet environment is much closer to the actual biological system than the dry state used in previous STXM and X-PEEM studies. The image sequences used in this analysis can be viewed at **http://unicorn.mcmaster.ca**. **Fig. 1** compares color coded composites of the component maps derived by SVD analysis of STXM image sequences [9]. The left hand result is derived from measurements when the sample had been dried after protein exposure. The right hand result is derived from measurements from the same sample after completely covering the section surface with a ~0.5 μm water layer (trapped between two silicon nitride windows). The sample is a TDI-based polyurethane containing SAN and PIPA aromatic filler particles [10] to which fibrinogen had been adsorbed (0.1 mg/ml buffer solution for 10 minutes at 25 C, followed by extensive rinsing without taking the sample through the solution-air interface).

Fig. 2 is a spectral deconvolution of the regions of strong fibrinogen signal. The characteristic protein signature at 288.2 eV is clearly seen. Quantitation using reference spectra [10] indicates the regions under the protein are mainly the soft-section dominated polyurethane matrix The relative amounts of PIPA:SAN:matrix are 1:3:9 from the fit of the summed signal in the strongly Fg regions to the model spectra (Fig. 2). However one can see from the composite component map (Fig. 1) that the protein is found mainly at the edges of the SAN particles which protrude above the surface due to pull-out effects in the cryotoming. The OD signal displayed in Fig. 2 was generated by referencing the transmitted intensity through the protein coated polymer section to the transmitted intensity through water and two 70 nm thick Si_3N_4 windows recorded from a region adjacent to the polymer section. When the incident flux reference without these two components is used for normalization, we deduce a water thickness of about 0.5 μm, corresponding to an additional optical density of 0.25 units.

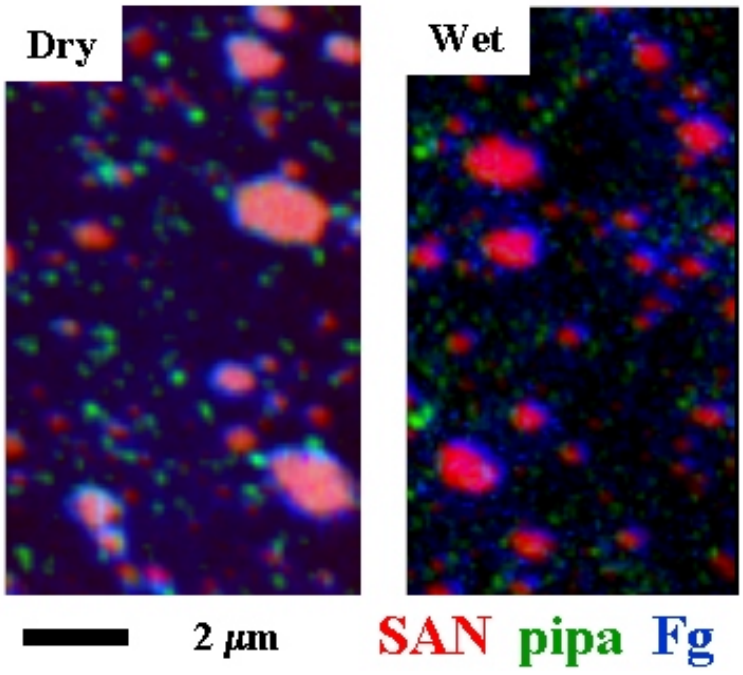

Fig. 1 Color coded composite of the component maps for two aromatic filler particles (**SAN**, **PIPA**) and a protein (**fibrinogen, Fg**) derived from STXM image sequence recorded from a dry and wet protein covered polymer. The left hand figure was measured in the dry state, while the right hand figure was imaged after an overlayer of water was added to the same section. The black areas are the soft-segment dominated polyurethane matrix.

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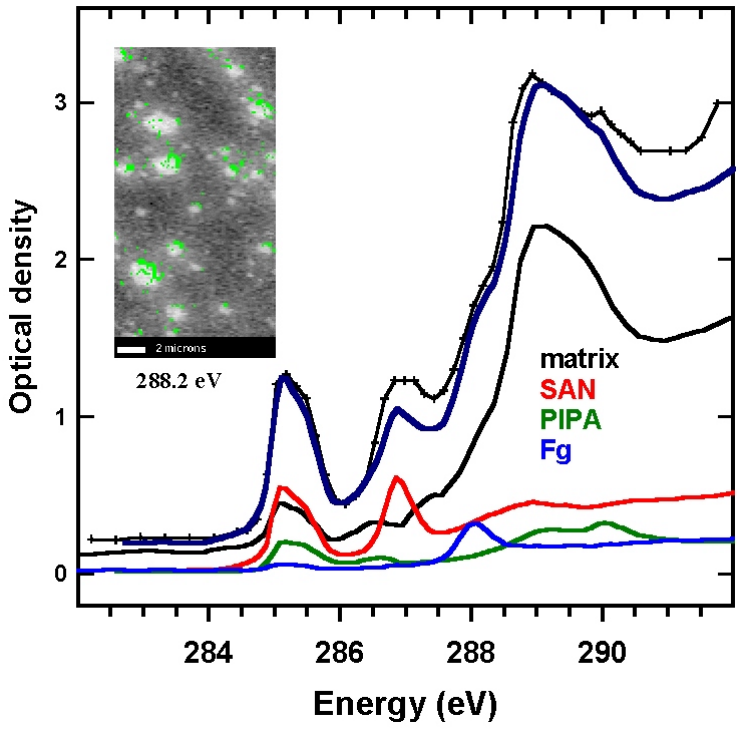

Fig 2 Spectral decomposition of the signal indicated in green on the 288.2 eV image. This corresponds to regions containing the most easily detected amounts of protein. The spectrum was isolated from the image sequence using a mask on the Fg component map for signals > 25 nm of Fg. An Fg protein is about 10x20 nm in size.